SYNOPSIS OF RESEARCH PROJECT

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8.	**Title of Research Project**	**:**	**GENETIC DIVERSITY ANALYSIS, ASSESSMENT OF G×E INTERACTION AND SCREENING OF BREAD WHEAT (*Triticum aestivum* L.) GENOTYPES FOR HEAT TOLERANCE USING SSR MARKERS**

9. Introduction

Wheat, commonly known as the **"King of Cereals"** plays an important role in worldwide agriculture because of its vast area under cultivation, high production capability and major contribution to international food trade. As a cool-season crop, wheat is essential for global food and nutritional security, serving as a staple food for nearly 40 per cent of the world's population and supplying about 20 per cent of total dietary calories (Bhutto *et al.,* 2016). The broad adaptability of wheat to a range of environmental conditions has enabled its extensive cultivation across the globe, with global production estimated to be nearly 842.2 million metric tonnes, wheat remains crucial in fulfilling global food requirements (Anonymous, 2025).

Wheat is a highly self-pollinated cereal crop belonging to the genus *Triticum* and the family *Poaceae*, with its origin traced to the Middle East (Lupton, 1987). In India, three major wheat species are under cultivation among these, bread wheat (*Triticum aestivum* L.) is one of the most widely cultivated species, occupying

approximately 86 per cent of the total wheat-growing area. Wheat is a hexaploid species with a chromosome number of 2n = 6x = 42 and is cultivated across all wheat-producing regions of the country. Durum or macaroni wheat (*Triticum durum* Desf.), a tetraploid species with 2n = 4x = 28 chromosomes, accounts for about 12 per cent of the wheat area and is primarily grown in northern states such as Punjab as well as in certain southern regions. Emmer wheat (*Triticum dicoccum* Schulb.), also tetraploid (2n = 4x = 28), covers nearly two per cent of the total wheat area and is mainly cultivated in Karnataka and parts of Gujarat (Das, 2008). The distribution of these species across different agro-climatic zones highlights wheat's adaptability and its significance in Indian agriculture.

Wheat is regarded as a highly versatile crop, serving as the raw material for a wide range of food products such as chapatis, bread, cakes, biscuits, pasta and numerous breakfast items. Its wide applicability in food processing is primarily attributed to the presence of gluten, a unique protein that enables dough to retain gas during fermentation, resulting in the characteristic light and airy texture of leavened breads a property largely exclusive to wheat among cereal crops. From a nutritional standpoint, wheat grains comprise approximately 60-68 per cent starch, 6-21 per cent protein, 1.5-2 per cent fat, 2-2.5 per cent cellulose, about 1.8 per cent minerals and various vitamins, making wheat a rich source of energy and essential nutrients (Das, 2008). Owing to this balanced nutritional composition, wheat continues to serve as a major staple food for billions of people worldwide.

India stands as the world's second-largest wheat producer, a status supported by its diverse agro-ecological zones that offer favourable conditions for successful wheat cultivation (Sharma and Sendhil, 2016). In India, during the 2024-25 crop season, wheat in India was cultivated on approximately 31,832.9 thousand hectares, producing about 113,292.4 thousand tonnes with an average productivity of 3559 kg/ha (Anonymous, 2025). In Gujarat, during 2024-25, wheat was cultivated on about 1281.6 thousand hectares, producing 3867.2 thousand tonnes with a productivity of 3017 kg/ha (Anonymous, 2025). The substantial production and rising productivity of wheat contribute significantly to India's food and nutritional security by stabilising domestic food supplies and helping to meet the dietary requirements of its vast population.

Wheat is highly vulnerable to elevated temperatures, which have shown an increasing trend in major wheat-producing regions (Alexander *et al.*, 2006). Although heat stress affects wheat throughout its growth cycle, the grain-filling stage is

particularly sensitive, where severe temperature stress can lead to yield losses up to 40 per cent (Hays *et al.,* 2007). High temperature stress occurring during this critical phase results in reduced grain weight, premature leaf senescence, shrivel grains, diminished starch deposition, modifications in starch-lipid composition and a decline in seed germination and vigour (Balla *et al.*, 2012). In response to these challenges, recent studies have emphasized the evaluation and development of heat-tolerant wheat genotypes. To mitigate these impacts, recent research has focused on screening and developing heat-tolerant wheat genotypes. By identifying and promoting varieties with higher resilience to late-season heat, the goal is to maintain wheat productivity and stability despite rising temperatures.

Heat stress is one of the major abiotic constraints limiting wheat growth, productivity and yield, particularly during the germination and grain-filling stages, as wheat is inherently adapted to cooler growing conditions. Consequently, its cultivation in warmer regions is largely confined to the winter season. The implications of rising temperatures for crop production and global food security have become a major concern worldwide (Reynolds, 2010). Large parts of South Asia's rice-wheat cropping systems are increasingly exposed to heat-stress environments, characterized by mean daily temperatures exceeding 17.5 °C even during the coolest month, described by Fischer and Byerlee (1991). Climate change induced shortening of winter duration and the earlier onset of high temperatures have particularly intensified heat stress in the eastern Gangetic plains of India, making these regions highly vulnerable.

The adverse impact of heat stress on wheat productivity in India was recognized as early as 1924, when Howard famously remarked that "**wheat growing in India is a gamble in temperature**." Terminal heat stress, especially during reproductive and grain-filling stages, has been identified as a major factor limiting the realization of potential wheat yields in the country (Nagarajan, 2005). Studies have shown that mean temperatures above 15-18 °C during anthesis significantly reduce grain yield (Wardlaw, 1994). In India, a significant proportion of wheat-growing areas, particularly in the Indo-Gangetic plains, is increasingly subjected to terminal heat stress under changing climatic conditions. Approximately 25-30 million hectares in tropical and subtropical regions are vulnerable (Kumar *et al*., 2023). Temperatures above 30-31°C during grain filling accelerate senescence and lower grain weight. Wheat yield declines by about 6-10% per 1°C rise in temperature during the reproductive stage (Mondal *et al*., 2013; Farhad *et al*., 2023) and under severe stress,

losses may exceed 30-50% (Asseng *et al*., 2015; Tuhin *et al*., 2025). Climate simulation models predict an average wheat yield reduction of 11.1 per cent under projected 2050 climate scenarios (Dubey *et al.,* 2020), while recent estimates suggest that each 1°C rise in global temperature results in approximately a 6 per cent reduction in wheat yield (Yadav and Bhatt, 2024).

Several yield-related traits such as chlorophyll content, canopy temperature depression, photosynthetic rate, biomass accumulation, thousand-grain weight and grain yield are markedly influenced by heat stress in wheat. Among these, grain-filling duration is widely recognized as an important indicator of heat tolerance (Fokar *et al.,* 1998). Elevated temperature conditions during the grain-filling stage accelerates crop maturity, shortens the grain development period and results in poor grain filling, ultimately leading to substantial yield reduction in wheat (Rane and Nagarajan, 2004). The heat stress tolerant varieties/genotypes can be identified by calculating heat susceptibility index (HSI) following field evaluation for a number of agronomic traits. Based on the method developed by Fischer and Maurer (1978), the heat susceptibility index (HSI) was determined for grain yield and other characteristics under heat stress (late sowing) and non-stress conditions (normal sowing).

Yield stability ratio (YSR) is a metric used to assess how consistently a plant variety produces yield across different environments, particularly under heat stress. A high YSR indicates greater stability, meaning the variety maintains its yield even when faced with heat stress (Lewis 1954).

The selection of superior genotypes for improved grain yield and other desirable agronomic traits is strongly supported by the assessment of genetic variability among different varieties and germplasm for yield and its contributing traits. The correlation between grain yield and its contributing traits often varies, while these traits are usually interrelated. Therefore, a clear understanding of the relationships among yield-related traits is essential for identifying the most effective trait combinations within a single genotype. Grain yield is a quantitative trait influenced by several components that act either directly or indirectly through other traits, making selection decisions challenging for breeders. To overcome this complexity, path coefficient analysis, originally proposed by Wright (1921), is employed to partition correlation coefficients into direct and indirect effects, thereby providing a clear interpretation of interrelationships among traits; this approach was later applied to plant breeding by Dewey and Lu (1959). Additionally, Smith (1936)

first applied Fisher's (1936) discriminant function analysis to develop selection indices, which estimate the relative contribution of individual yield components and help to assess the combined effect of multiple traits on yield improvement.

In transgressive breeding programmes, the identification of genetically diverse parents for hybridization relies on the proper classification of breeding materials using appropriate selection criteria. Among the various methods available, the Mahalanobis D^2 statistic is considered as one of the most reliable techniques for measuring genetic divergence (Mahalanobis,1936). Genetic diversity plays a vital role in plant breeding, as crosses between genetically distant parents generally exhibit higher heterosis compared to those involving closely related lines and often generate broad genetic variability in the segregating generations (Arunachalam, 1981).

Environmental factors exert a considerable influence on wheat grain yield; however, the relative contribution of environmental effects, genetic factors and genotype × environment (G × E) interactions to yield variation is often unclear. Hence, the development of strategies for increasing grain yield requires detailed knowledge of the magnitude and nature of G × E interactions. Such interactions may cause changes in genotype ranking across environments, differences in performance scale among environments, or a combination of both. When genotypes respond differently under varying environmental conditions, G × E interaction becomes a major challenge in crop improvement programmes, often compelling breeders to develop either broadly adapted or environment-specific genotypes. The importance of G × E interaction lies in its ability to provide insight into cultivar responses across environments and to aid in the evaluation of performance stability of breeding materials. Both high yield potential and stable performance under specific environmental conditions are essential to ensure sustained productivity (Yagdi, 2002). Terminal heat stress represents one of the most critical environmental constraints affecting wheat productivity in many regions. In the present study, efforts are directed toward identifying wheat genotypes that combine superior yield performance with stability under heat stress conditions. Although G × E interactions complicate the selection of superior genotypes, their interpretation can be enhanced through appropriate statistical approaches. Accordingly, stability analysis was performed using the models proposed by Eberhart and Russell (1966) and the AMMI (Additive Main Effects and Multiplicative Interaction) model originally developed by Gauch (1988) and further elaborated by Gauch and Zobel (1996), along with later applications described by Farshadfar *et al.* (2011).

A fundamental understanding of genetic diversity and population structure of breeding material is required prior to initiating any crop improvement programme (Van Inghelandt *et al.,* 2010). This knowledge helps in assessing diversity, inheritance pattern, extent of heterosis (Qi-Lun *et al*., 2008). Genetic diversity and population structure are commonly assessed using molecular markers, including restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA (RAPDs), amplified fragment length polymorphisms (AFLPs), single nucleotide polymorphisms (SNPs), sequence-related amplified polymorphisms (SRAPs) and simple sequence repeats (SSRs), which offer several advantages over phenotypic markers despite certain limitations. In contrast to morphological traits, molecular markers are largely unaffected by environmental factors and typically exhibit Mendelian inheritance patterns (Legesse *et al*., 2007). In studies focused on heat tolerance, researchers often employ gene-specific SSR markers linked with heat-responsive traits. These markers comprise short, repetitive DNA sequences that exhibit length polymorphism, enabling the detection of genetic variation within and among populations and facilitating the identification of genomic regions linked to heat tolerance.

10. Practical utility of the research project

Heat-tolerant genotypes identified using the Heat Susceptibility Index (HSI) and Yield Stability Ratio (YSR), evaluate a genotype's capacity to sustain yield under high-temperature stress in comparison with normal conditions. Lower HSI values denote greater tolerance to heat stress, whereas higher YSR values reflect superior yield stability under stress environments.

Estimation of genetic parameters such as genotypic and phenotypic coefficients of variation, heritability and genetic advance is valuable for assessing the extent of heritable variability among the breeding lines under diverse environments, including late-sown conditions where crops are exposed to elevated temperatures during the reproductive stage.

Correlation analysis is important for determining the nature and strength of associations between various developmental and yield-related traits and seed yield under different environments. The information obtained from such studies is valuable for plant breeders in identifying key traits to be emphasized in selection programmes, particularly for developing wheat genotypes with enhanced heat tolerance.

Path coefficient analysis enables breeders to assess the direct and indirect effects of various quantitative traits on grain yield, thereby facilitating more informed selection decisions.

Selection based on a selection index has been shown to be more effective than simple directional selection for improving yield. Accordingly, the use of selection indices in the form of discriminant functions, as proposed by Smith (1936), is valuable for identifying optimal combinations of yield-contributing traits and serves as a useful tool for wheat breeders in formulating effective selection criteria.

The phenotypic performance of a genotype is governed by its genetic constitution, the prevailing environment, and the interaction between these factors. The presence of significant genotype × environment interaction indicates differential responses of genotypes to environmental changes, suggesting that a genotype performing optimally in one environment may not exhibit similar performance under different conditions. Stability analysis therefore assists in identifying broadly adaptable genotypes and in understanding their adaptive behaviour across varying environments.

Assessment of genetic diversity among bread wheat genotypes using gene-specific SSR markers will facilitate the rapid identification of genotypes possessing heat tolerance.

11. Objectives

Based on the foregoing background, the present investigation entitled "Genetic diversity analysis, assessment of G×E interaction and screening of bread wheat (*Triticum aestivum* L.) genotypes for heat tolerance using SSR markers" will be carried out with the following objectives:

1. To estimate genetic variability parameters and to work out genotypic and phenotypic correlations in relation to heat tolerance
2. To determine the direct as well as indirect effects of different yield components on grain yield using path coefficient analysis
3. To find out the extent of genetic diversity among bread wheat genotypes through Mahalanobis D^2 technique
4. To calculate discriminant function for constructing and identifying the most effective selection indices
5. To find out the nature and magnitude of G × E interaction and workout stability parameters for grain yield and important yield components

6. To study the molecular diversity on the basis of heat tolerance gene specific Simple Sequence Repeats (SSRs) markers.

12. Review of Literature

12.1 Identification of heat tolerant wheat genotypes

Heat or thermal stress has emerged as a major plant breeding objective in bread wheat, one of the most important staple cereal crops, and its significance is increasing progressively due to global climate change. High temperature stress accelerates the phenological development of wheat plants, thereby influencing growth, yield, and related traits. Consequently, it is essential to examine and compare the assessment of wheat genotypes under heat-stress and non-stress environments. Several physiological and yield-based parameters, such as membrane thermostability (MT), canopy temperature depression (CTD), stomatal conductance, chlorophyll fluorescence, heat sensitivity index (HSI) and yield stability ratio (YSR), serve as effective screening tools for identifying heat-tolerant wheat genotypes.

Rehman *et al.* (2021) evaluated the morpho-physiological responses of two spring wheat cultivars (Millet 11 and Punjab 11) and two advanced breeding lines (V 07096 and V 10110) under terminal heat stress imposed through late sowing. The early-maturing cultivar Millet 11 was used as a heat-tolerant check. Late sowing caused a reduction in spike length (13%), grains per spike (10%), thousand-grain weight (13%) and biological yield (15-20%) compared with normal sowing. However, an increase in productive tillers per plant (19-20%) and grain yield (9%) was observed under late planting. Punjab 11 exhibited delayed maturity and better agronomic performance relative to the early-maturing, heat-tolerant Millet 11. Under terminal heat stress, the advanced lines maintained lower canopy temperature during grain filling and recorded higher chlorophyll *a* (20%) and chlorophyll *b* (71-125%) contents at anthesis. The genotype Millet 11 showed the highest accumulation of stem water-soluble carbohydrates at anthesis under heat stress conditions. Higher chlorophyll content, associated with stay-green behaviour, sustained photosynthetic activity and cooler canopies under late sowing, which ultimately contributed to improved grain yield.

Kajla *et al.* (2022) investigated the impact of terminal heat stress caused by late sowing on grain yield and its associated traits in ten bread wheat genotypes and forty-five F_1 hybrids. Based on the heat susceptibility index, the genotypes RAJ 3077, RAJ 1482 and RAJ 3777 were identified as highly tolerant to elevated temperature conditions compared to the other entries. Among the crosses, RAJ 4079 × RAJ 3765,

RAJ 3077 × PBW 550, WH 1021 × DBW 90, RAJ 1482 × HD 3086 and RAJ 4238 × WH 1021 exhibited significantly superior tolerance for grain yield per plant under late-sown environments, indicating their potential usefulness for predicting and selecting thermo-tolerant wheat genotypes.

Patel *et al.* (2023) employed the heat susceptibility index (HSI) and yield stability ratio (YSR) to identify heat-tolerant bread wheat genotypes. Based on pooled data over two years, eight genotypes AKAW 4901, UAS 375, K 1317, WH 1127, HTW 11, WH 1105, HIKK 09 and DBW 14 were classified as heat tolerant. These genotypes recorded low heat susceptibility (HSI < 0.50) along with high yield stability (YSR > 83%), indicating minimal reduction in grain yield under heat stress conditions.

Baranwal *et al.* (2024) evaluated a panel of 225 wheat genotypes under normal (timely sown) and terminal heat stress (late sown) conditions. Heat susceptibility index (HSI) values were estimated for grain filling duration (GFD), 1000 grain weight (TGW) and yield per plot (YPPT). The HSI for GFD varied from 0.36 in genotype 30 (GID: 7933202) to 2.51 in genotype 54 (GID: 7933656), while for TGW it ranged from -0.84 in genotype 44 (GID: 7933473) to 2.66 in genotype 36 (GID: 7933333). Similarly, HSI for YPPT ranged from -0.49 in genotype 37 (GID: 7933334) to 1.93 in genotype 49 (GID: 7933509). Genotypes 88, 74 and 66 exhibited low to moderate HSI for GFD, TGW and YPPT, respectively. Based on percentage reduction in yield per plot under heat stress, genotype 19 (GID: 7933096) recorded the minimum yield reduction (-23.00%) along with negative HSI values for YPPT (-0.49) and TGW (-0.60), followed by genotype 224 (DBW 14), which showed a yield reduction of 19.21% with HSI values of 0.41 for YPPT and -0.56 for TGW.

Kiranakumara *et al.* (2024) estimated heat stress indices, including the heat susceptibility index (HSI) and heat tolerance index (HTI), to assess thermo-tolerance in wheat genotypes. Among the parental lines, HD 2864 was identified as heat susceptible, whereas DBW 14 exhibited heat tolerance. Among the check varieties, QCSN 35 and QUAIU were categorized as heat susceptible, while HD 3090, NI 5439, RAJ 3765, WH 730 and HD 2932 were classified as heat tolerant. Furthermore, evaluation of F_4 progenies derived from the cross HD 2864 × DBW 14 revealed that 45 families were heat tolerant and 10 families were moderately tolerant based on HSI values.

Labonno *et al.* (2025) evaluated 36 mutant wheat lines under controlled and heat-stress conditions using morpho-physiological indices to identify heat-tolerant genotypes. Significant variation was observed among 15 traits across both environments. Pigment-related traits and chlorophyll fluorescence (Fv/Fm) exhibited strong positive associations with root and shoot biomass, while showing negative relationships with relative water content and cell membrane stability. The identified heat-tolerant mutant lines were suggested as promising genetic resources for enhancing wheat productivity under rising temperature conditions.

12.2 Genetic variability

The existence of genetic variability forms the foundation of any crop improvement programme; however, the rapid erosion of locally adapted genetic resources has led to a widespread reduction in variability, highlighting the need for its conservation. Knowledge of the extent of genetic variability within a population, along with estimates of variability parameters such as heritability, is essential for formulating an effective breeding strategy. The variation observed among homozygous genotypes or populations is regarded as freely exploitable variability and can be effectively utilized to achieve genetic improvement. When combined with information on heritability and expected genetic advance, such estimates provide valuable guidance for designing efficient crop breeding programmes.

Poudel *et al.* (2021) evaluated agro-morphological variability among wheat genotypes under both normal and heat-stress conditions. Under normal conditions, moderate genotypic and phenotypic coefficients of variation (GCV and PCV) were observed for spike weight, thousand-grain weight, grains per spike and grain weight per spike. In contrast, grain yield exhibited high GCV and PCV under heat stress. High heritability coupled with high genetic advance as a percentage of mean was calculated for thousand-grain weight and grain yield under normal conditions, while under heat stress, these parameters were prominent for thousand-grain weight, grains per spike and grain weight per spike.

Regmi *et al.* (2021) estimated genetic parameters in different wheat genotypes to assess variability and inheritance of yield-related traits. Analysis of variance indicated significant differences among genotypes for all traits studied, except spike length, indicating the presence of substantial genetic variability for most characters. Across traits, phenotypic coefficients of variation (PCV) were generally higher than genotypic coefficients of variation (GCV), suggesting environmental influence. Large

disparities between PCV and GCV for spike length, grains per spike, grain weight per spike, grain yield, thousand-grain weight and harvest index highlighted the considerable effect of environment on these traits. Moderate to high heritability with high genetic advance was observed for days to flag leaf emergence, panicle length, thousand-grain weight, grain yield and harvest index, suggesting the major role of additive gene action. Based on grain yield performance, genotypes WK 2525, WK 2437 and WK 2506 were identified as promising.

Kumar *et al.* (2024) evaluated 26 physio-morphological and yield-related traits in wheat under both normal and heat-stress environments. High genotypic and phenotypic coefficients of variation were recorded for plant waxiness (8.897 and 11.943), followed by leaf rolling (6.390 and 12.240). Protein content exhibited the highest heritability, whereas relatively low heritability estimates were observed for grains per plant, leaf rolling, spike length, grain filling duration, spikelets per spike, and grain length. Moderate genetic advance as a percentage of mean was noted for plant waxiness, while the lowest genetic advance was recorded for chlorophyll content, canopy temperature depression, days to 50 per cent heading, flag leaf length, and harvest index.

Chandramaniya *et al.* (2025) evaluated genetic variability for grain yield and yield related morphological, physiological and quality parameters in wheat under two contrasting environments. Comparative performance of genotypes indicated that heat stress exerted a significant adverse effect on most traits, leading to reduced grain yield, while simultaneously improving flour quality parameters such as wet gluten content (WGC), protein content (PC) and sedimentation value (SV). Traits including effective tillers per plant, spike length, grains per spike, biological yield per plant, harvest index, grain yield per plant, grain filling rate and duration, and stem solidness exhibited moderate to high genotypic and phenotypic coefficients of variation along with high heritability and moderate to high genetic advance as a percentage of mean under both environments. These results suggested the predominance of additive gene action and high selection potential for these traits.

12.3 Correlation analysis

The knowledge of the correlation between yield and its component traits is of great importance to plant breeders, as it provides a fundamental basis for effective selection. Yield components often show varying degrees of interrelationship, exhibiting both positive and negative associations among themselves as well as with

yield. Hence, understanding the nature and magnitude of correlations among characters is essential for assembling an optimal combination of yield-contributing traits within a single genotype.

Poudel *et al.* (2021) assessed the correlation among agro-morphological traits in wheat genotypes under both normal and heat stress conditions. In both environments, spike weight exhibited a positive association with grain yield.

Das *et al.* (2024) estimated correlation coefficients for eleven morphological and physiological traits under normal and terminal heat stress conditions. Under both environments, grain yield per plant exhibited a positive and significant association with number of spikelets per spike, number of tillers per plant, grains per spike, grains per plant, 1000-grain weight and chlorophyll content, while showing a significant negative correlation with plant height and days to 50 per cent flowering. The strongest positive correlations were recorded for grains per plant and chlorophyll content, whereas days to 50 per cent flowering showed the highest negative association with yield. Under terminal heat stress, canopy temperature was negatively correlated with grain yield and with all the studied traits except plant height. Additionally, days to 50 per cent flowering showed a negative correlation with all traits under heat stress conditions.

Kumar *et al.* (2024) evaluated twenty-six physio-morphological and yield-related traits under both non-stress and heat stress environments. The study revealed that grain yield per plant had a significant positive genotypic correlation with grain length (0.712), harvest index (0.629), leaf rolling (0.463), days to 50 per cent heading (0.274), grain filling period (0.402) and protein content (0.3767), while a significant negative genotypic association was observed with plant height (-0.461). At the phenotypic level, grain yield per plant showed significant positive correlations with harvest index (0.456) and protein content (0.235) and a significant negative association with plant height (-0.205).

Poudel *et al.* (2024) conducted a correlation analysis in wheat under heat stress conditions. The study revealed a strong positive association between number of spikelets per spike and spike weight, while thousand-grain weight was also strongly and significantly positively correlated with spike weight. Grain yield showed a substantial positive correlation with number of tillers per square meter. Moreover, yield was positively associated with spike length, plant height, spike weight, number of spikelets per spike and 1000-grain weight.

Rajput *et al.* (2025) investigated the relationship between grain yield and its component traits in forty-eight diverse bread wheat genotypes. Correlation analysis at both genotypic and phenotypic levels revealed that grain yield per plant exhibited highly significant positive associations with number of tillers per plant, number of grains per spike, 1000-grain weight, harvest index and spike length, indicating the importance of these traits as key determinants of yield and their potential utility for yield enhancement in selection for yield improvement in bread wheat.

12.4 Path coefficient analysis

Grain yield is a complex trait resulting from the combined effect of several interrelated component characters. Understanding these interrelationships helps in determining the magnitude and direction of their associations. However, reliance on correlation analysis alone may be misleading when multiple traits influence yield simultaneously. Hence, path coefficient analysis is an effective tool for partitioning the total association of yield-related traits into their direct and indirect effects.

Devesh *et al.* (2021) conducted correlation and path coefficient analyses for nineteen traits in wheat. Path analysis revealed that biological yield per plant exerted the highest positive direct effect on grain yield per plant, followed by harvest index, while wet gluten percentage showed a low positive direct effect. In contrast, number of spikelets per spike and plant height exhibited negligible negative direct effects on grain yield.

Poudel *et al.* (2021) conducted path coefficient analysis for agro-morphological traits of wheat genotypes under both normal and heat stress conditions. Under normal conditions, spike weight showed a high positive direct effect on grain yield at the genotypic level, while weight of grains per spike exerted a high positive direct effect at the phenotypic level. Under heat stress conditions, spike weight continued to exhibit a strong positive direct effect on grain yield at the genotypic level, whereas weight of grains per spike and plant height showed high positive direct effects at the phenotypic level.

Mahpara *et al.* (2024) conducted path coefficient analysis to examine the direct and indirect effects of yield-related traits on grain yield in bread wheat genotypes. The analysis indicated that flag leaf area, plant height, spike length, number of spikelets per spike, and grain weight per spike exerted positive direct effects on grain yield, whereas the remaining traits showed negative direct effects. Additionally, flag leaf area and number of grains per spike contributed positive effect on grain yield per plant through their indirect effects.

Poudel *et al.* (2024) conducted path coefficient analysis in wheat under heat stress conditions to determine the direct and indirect effects of yield-related traits on grain yield. The study revealed that number of tillers per meter, followed by total grain weight and plant height, had strong positive direct effects on grain yield. Traits such as spike weight, spike length, thousand-grain weight, and number of spikelets per spike also contributed positively. Indirect effects varied among traits, with spike weight and thousand-grain weight generally showing positive associations via spikelets per spike, while plant height and tillers per meter exhibited some negative indirect effects through other traits.

12.5 Genetic diversity based on D^2 statistics

In any hybridization programme, the selection of genetically diverse parents is of paramount importance, as crossing such parents increases the likelihood of obtaining superior hybrids or desirable segregants in later generations. Several multivariate statistical approaches have been developed to quantify divergence among populations, including the coefficient of racial likeness (Pearson, 1926), multiple regression analysis (Hotelling, 1936), discriminant function analysis (Fisher, 1936) and the D^2 statistic (Mahalanobis, 1936). Among these methods, the D^2 statistic is most widely employed, as it provides a precise quantitative measure of genetic divergence among biological populations and has been extensively utilized for assessing genetic diversity.

Santosh *et al.* (2019) evaluated genetic diversity among 32 bread wheat genotypes under late-sown conditions to assess tolerance to terminal heat stress. The analysis of genetic divergence revealed that grain yield per plot contributed the highest proportion to total genetic variability (47.98%), whereas harvest index contributed the least (0.81%). Cluster analysis showed that clusters I and III contained the maximum number of genotypes (14 each), while clusters II, IV, V and VI were contained a single genotype each. The highest intra-cluster distance was observed in cluster III (703.41), suggesting substantial genetic variability within this group. The maximum inter-cluster distance occurred between clusters V and VI (7190.38), indicating wide genetic divergence between these clusters. Additionally, nineteen genotypes were identified as heat tolerant.

Gerema *et al.* (2020) evaluated 180 bread wheat genotypes to assess the magnitude of genetic diversity and the correlation among yield and its associated traits. Based on Mahalanobis D^2 divergence analysis, the entire germplasm set was classified into ten different clusters. Among these, clusters IV and IX exhibited the

maximum inter-cluster distance, indicating considerable genetic divergence. Therefore, crosses involving genotypes from these two clusters are likely to generate superior segregating populations due to their wide genetic base.

Baranwal *et al.* (2024) assessed 225 wheat genotypes under contrasting environments, namely heat stress induced by late sowing and normal timely sowing, to estimate genetic divergence using Mahalanobis D^2 analysis for sixteen traits. The genotypes were classified into seven clusters, with cluster V being the largest (51 genotypes), followed by clusters I, II, IV, VI and VII containing 36, 36, 21, 44 and 26 genotypes, respectively, while cluster III was the smallest with only 11 genotypes. The highest mean grain yield per plot was recorded in cluster II (283.33 g), followed by cluster III (198.67 g). Intra-cluster distances were maximum in clusters III (3.353), IV (3.311) and I (3.152), indicating substantial variability within these clusters, whereas lower intra-cluster distances were observed in clusters VI (2.358) and V (2.882), suggesting comparatively less variability. The greatest inter-cluster distance was observed between clusters III and VII (6.958), followed by clusters III and V (6.499) and clusters III and I (6.341), reflecting wide genetic divergence, while the minimum inter-cluster distance occurred between clusters VII and V (2.533), indicating close genetic relatedness among these clusters.

Santosh and Jaiswal (2024) analyzed genetic diversity in 32 bread wheat genotypes to identify terminal heat tolerance under extremely late-sown conditions. Data were recorded for sixteen agronomic traits along with three physiological parameters. Cluster analysis grouped the genotypes into six clusters, with cluster III comprising the largest number of genotypes, followed by clusters I, II, IV, V and VI. The highest inter-cluster distance was observed between clusters IV and VI, whereas the lowest was recorded between clusters I and II, indicating close genetic similarity. Cluster III exhibited the highest intra-cluster distance, reflecting maximum genetic variability among its genotypes, while cluster VI showed the least intra-cluster distance. Five genotypes were identified as heat-tolerant. The study suggested that selecting superior genotypes from genetically diverse clusters could be effectively employed in future breeding programs to enhance yield and physiological performance of bread wheat under terminal heat stress caused by extremely late sowing.

Yasin *et al.* (2024) evaluated the genetic diversity in 40 bread wheat lines using Mahalanobis D^2 statistics. Based on intra- and inter-cluster distances, the genotypes were grouped into ten distinct clusters. The highest intra-cluster distance

was recorded in cluster X, indicating greater variability within this group, while the maximum inter-cluster divergence was observed between clusters VI and X, followed by clusters IX and X. Most wheat lines showed substantial variation with respect to their cluster means, suggesting meaningful differences in average performance among clusters. Grain yield per plant contributed the most to total genetic divergence, followed by spike length, effective tillers per plant, thousand-grain weight and plant height. In addition, traits such as protein content, number of grains per spike, thousand-grain weight, harvest index, spike length and effective tillers per plant exhibited high heritability along with considerable genetic advance as a percentage of the mean, indicating strong prospects for improvement through selection.

12.6 Selection indices

Grain yield is a complex, polygenic trait governed by several interacting factors, and direct selection based solely on yield is often inefficient because of the effect of multiple component characters. Therefore, to achieve effective improvement in grain yield, it is essential to evaluate the relative efficiency of selection through the discriminant function technique rather than relying on straight selection. The discriminant function approach, implemented through appropriate selection indices, was originally developed by Fisher (1936) and was first successfully applied in plant breeding selection programs by Smith (1936).

Shah *et al.* (2016) developed selection indices using 52 bread wheat genotypes by constructing a total of sixty-three indices that incorporated grain yield per plant along with its five contributing traits. Among these, the selection index comprising grain yield per plant, hundred-grain weight, days to maturity, harvest index and number of effective tillers per plant exhibited the highest genetic advance (155.77 g) and relative efficiency (1867.41%). The next most effective index was based on grain yield per plant, biological yield per plant, hundred-grain weight, days to maturity and harvest index, which recorded a genetic gain of 53.85 g and a relative efficiency of 1856.78%.

Patel and Raval (2018) developed selection indices for 48 bread wheat genotypes grown under late-sown conditions using the discriminant function approach. A total of thirty-one indices were formulated by incorporating grain yield per plant and its four component traits. The selection index comprising three traits biological yield per plant, grain yield per plant and number of productive tillers per plant recorded the highest relative efficiency and genetic gain, followed by the index

that included four traits, namely biological yield per plant, grain yield per plant, plant height and number of productive tillers per plant. These findings highlighted the usefulness of such selection indices in identifying high-yielding bread wheat genotypes.

Reddy and Babariya (2020) evaluated 48 bread wheat genotypes for the construction of selection indices using the discriminant function technique. A total of sixty-three indices were formulated by incorporating grain yield per plant along with its five contributing traits. The study revealed that inclusion of more characters in the selection index enhanced selection efficiency. Among the indices, the one comprising grain yield per plant, number of grains per main spike, grain weight per main spike and biological yield per plant exhibited the maximum genetic gain and relative efficiency.

Detroja *et al.* (2024) developed selection indices for 50 bread wheat genotypes using the discriminant function approach. A total of thirty-one selection indices were formulated by considering all possible combinations of grain yield per plant and four yield-contributing traits, namely number of grains per main spike, grain weight per main spike, biological yield per plant and harvest index. Among these, the index comprising five traits grain yield per plant, number of grains per main spike, grain weight per main spike, biological yield per plant and harvest index recorded the highest genetic gain (26.99) and relative efficiency (807.60%). This was followed by an index based on four traits, including grain yield per plant, number of grains per main spike, biological yield per plant and harvest index, which showed high genetic advance (26.29) and relative efficiency (786.55%). The highest relative efficiency per character was observed for number of grains per main spike (433.25%), followed by the combination of number of grains per main spike and biological yield per plant (303.58%). These findings suggested that greater importance should be given to these traits while selecting high-yielding wheat genotypes.

Mangroliya *et al.* (2024) developed selection indices for 50 bread wheat genotypes under timely sown conditions using the discriminant function technique. In total, thirty-one selection indices were formulated by incorporating grain yield per plant along with four yield-related traits. The study revealed that selection efficiency increased with the inclusion of a higher number of characters in the index. Among the indices, the one emphasizing two traits biological yield per plant and harvest index proved to be the most effective in identifying high-yielding genotypes. The results

highlighted the need to give maximum emphasis to these traits while selecting for yield improvement in bread wheat.

Chandramaniya *et al.* (2025) evaluated 48 bread wheat genotypes subjected to heat stress conditions in the South Gujarat agro-climatic zone using 21 agro-physiological and quality traits to develop effective selection indices. Using discriminant function analysis, 31 selection indices were formulated, among which indices combining chlorophyll content index with harvest index and grain yield per plant with harvest index exhibited the highest relative efficiency per trait. The study highlighted the superiority of selection index-based selection over direct grain yield selection for identifying heat-tolerant wheat genotypes.

12.7 Genotype × environment interaction and stability analysis

The phenotypic expression of an individual is governed by its genotype, the environment, and their interaction. Among these, genotype × environment (G × E) interaction is particularly important, as significant interactions indicate differences in the relative performance of genotypes, often resulting in changes in genotype ranking across environments (Fernandez, 1991). Based on the nature of environmental influence, G × E interactions are categorized into qualitative and quantitative types (Peto, 1982). Qualitative G × E interactions pose greater challenges in selection, as they hinder the identification of consistently superior genotypes across diverse environments.

Karkaji *et al.* (2023) evaluated 25 wheat genotypes across three growing seasons to assess genotype × environment interaction for grain yield. Combined analysis of variance using the AMMI model revealed a significant GEI effect on grain yield and the relative contribution of genotype, GEI and the first interaction principal component confirmed the suitability of the AMMI approach for data interpretation. GGE biplot analysis delineated five distinct mega-environments, identifying Varan, Saein, Sabalan, Sardari-101, F130-L-1-12/LAGOS, PYN/BAU//BONITO and SUBEN-7 as the highest-yielding genotypes in mega-environment I, which encompassed environments E1 to E6. Stability assessment based on both AMMI and GGE biplot methods highlighted SUBEN-7, PYN/BAU//BONITO, Sabalan, Sardari-101, F130-L-1-12/LAGOS and Saein as stable and high-performing genotypes, making them promising genetic resources for wheat improvement programs across diverse environments.

Patel *et al.* (2023) assessed genotype × environment interaction and yield stability in 52 bread wheat genotypes grown under normal (E1 and E3) and late-sown (E2 and E4) conditions over two years. Stability analysis based on pooled analysis of variance revealed highly significant effects of genotypes and environments for all traits, genotype × environment interactions were also significant for all characters, confirming differential genotype responses across environments. Genotypes AKAW 4901, K 1317 and UAS 375 were identified as stable due to their high grain yield per plant along with non-significant regression coefficients and deviations from regression. AKAW 4901 additionally exhibited stability for biological yield per plant and showed favourable-environment stability for productive tillers per plant, spikelets per main spike, grains per main spike and flag leaf area. K 1317 was stable for thousand-grain weight, with favourable-environment stability for productive tillers per plant and biological yield and unfavourable-environment stability for grain filling period, plant height, spike length, spikelets per main spike and grains per main spike. UAS 375 also showed stability for biological yield per plant, along with favourable-environment stability for days to 50% heading and unfavourable-environment stability for grain filling period, spike length and grains per main spike.

Bhandari and Poudel (2024) assessed the yield performance and stability of elite wheat genotypes under irrigated and heat stress conditions, generating six distinct test environments. Combined analysis of variance showed that heat stress had a highly significant effect ($p < 0.01$) on all quantitative traits. The "which-won-where" (WWW) biplot analysis identified BL 4407, NL 1384 and NL 1346 as the top-performing genotypes under irrigated conditions, whereas BL 4407, NL 1384 and NL 1381 were superior under heat stress. The WWW model further indicated that genotypes NL 1369, NL 1386, and NL 1376 exhibited high stability across both environments. Grain yield was strongly associated with days to booting, plant height, spike length, ten-spike weight and thousand-kernel weight.

Khare *et al.* (2024) investigated genotype × environment interactions in wheat using both univariate and multivariate analytical approaches. Ten wheat cultivars were evaluated across two years under timely and late-sown conditions, including grain yield per plant and grain filling rate serving as the primary traits for stability assessment. The results revealed significant effects of genotype, environment and their interaction across all test conditions. Genotypes JW 3288, L 8 and L 13 consistently exhibited superior grain yield performance, while L 11 and L 13 showed

high stability for grain filling rate. Multivariate methods, particularly the AMMI and GGE biplot models, demonstrated greater accuracy and effectiveness than the linear regression approach. Overall, genotype L 13 emerged as the most stable and high-performing cultivar across environments and planting conditions based on comprehensive stability analyses.

Mullualem *et al.* (2024) examined genotype × environment interaction and yield stability of 12 bread wheat genotypes across three environments over two years using AMMI and GGE biplot analyses. The AMMI biplot identified Tay as a high-yielding and widely adapted genotype, while Ogolcho and Honqolo exhibited high stability with minimal environmental interaction based on their IPCA1 scores. GGE biplot polygon analysis indicated that Tay recorded the highest grain yield in environment E3, which was also identified as the most representative and discriminative environment. Stability assessment using different indices showed that Balcha, Honqolo and Ogolcho were the most stable genotypes according to the AMMI stability value, whereas the genotype selection index ranked Honqolo as the most stable overall. Although Tay showed superior yield performance, it was classified as unstable by ASV and ranked third for stability based on GSI.

Desheva *et al.* (2025) reported that wheat grain quality is a multifaceted trait influenced by several components whose effects differ across environments. The study investigated the impact of genotype, environment and genotype × environment interaction on quality attributes of 16 wheat genotypes and analyzed the relationships among these traits. Two-way ANOVA indicated highly significant ($p \leq 0.001$) effects of all three sources of variation on grain physical characteristics. Genotype was the predominant factor influencing thousand kernel weight, test weight, vitreousness, sedimentation value and dry gluten content, whereas genotype × environment interaction accounted for a greater proportion of variability in crude protein and lysine content. Nearly equal contributions of genotype and genotype × environment interaction were observed for wet gluten content and bread-making strength index, while variation in gluten weakness was largely governed by genotype × environment interaction.

12.8 Genetic diversity based on molecular markers

Molecular markers are fundamental tools in genomic research and have wide applications in wheat improvement programs. Several PCR-based marker systems, including RAPD, AFLP, RFLP, microsatellites (SSRs) and single nucleotide

polymorphisms (SNPs), have been effectively employed for wheat germplasm fingerprinting and the analysis of genetic diversity.

Haque *et al.* (2021) assessed drought tolerance in wheat using morphological traits and SSR markers under pot conditions at 30% and 100% field capacity from the third leaf stage to maturity. Durum, Pavon-76, BARI Gom-25 and BARI Gom-33 were classified as moderately tolerant, BARI Gom-24, BINA Gom-1 and BARI Gom-20 as susceptible, while Sonalika, Sourav and BARI Gom-28 showed minimal yield reduction and were identified as tolerant. Molecular analysis of 26 genotypes using 13 drought-linked SSR markers detected 73 alleles (5.62 alleles per locus), with high genetic diversity (Nei's = 0.69; PIC = 0.73). Genetic similarity ranged from 0.15 to 1.00, and cluster analysis grouped tolerant and moderately tolerant genotypes together, particularly Durum, Pavon-76, Sonalika and Sourav, confirming their drought tolerance through both morphological and molecular evidence.

Devi *et al.* (2023) assessed the genetic diversity of 80 bread wheat genotypes using 43 polymorphic SSR markers. The analysis detected 84 alleles, with an average of three alleles per locus. Several primers, including Xgwm129, Xgwm131, TaGST, CFA2147, Xwmc48, Xbarc1165 and Xwmc169, were particularly informative due to their high polymorphism information content, with PIC values ranging from 0.26 to 0.82. Cluster analysis based on SSR data divided the genotypes into two major clusters comprising 35 and 45 genotypes, respectively, with genetic dissimilarity coefficients varying from 0.14 to 0.42. Population structure analysis using the STRUCTURE program further divided the genotypes into four distinct sub-populations.

Turkoglu *et al.* (2023) examined molecular diversity in bread wheat using 120 microsatellite markers. The analysis identified 651 polymorphic alleles for assessing genetic diversity and population structure, with allele numbers per locus ranging from 2 to 19 and an average of 5.44 alleles. Polymorphism information content values varied from 0.03 to 0.92, with an average of 0.43, while gene diversity ranged from 0.03 to 0.92 with an average of 0.46. The average effective number of alleles, Nei's genetic diversity and Shannon's information index were estimated as 1.190, 1.049 and 0.168, respectively. The highest genetic distance was observed between genotypes Mufitbey and Aksel 2000.

Patel *et al.* (2024) aimed to identify heat-tolerant bread wheat genotypes using 14 heat stress–associated SSR markers representing the A, B and D genomes. Among

the 52 wheat genotypes analyzed, these markers detected 30 polymorphic alleles. The polymorphism information content values of the SSR primers varied from 0.20 to 0.49, indicating moderate levels of informativeness. Cluster analysis based on Jaccard's similarity coefficients revealed considerable genetic variation, with the lowest similarity (5%) observed between genotypes AKAW 4901 and DBW 246, while the highest similarity (88%) occurred between DBW 179 and WH 1105.

Chandramaniya *et al.* (2025) assessed molecular diversity for terminal heat stress tolerance among twenty wheat genotypes using 25 heat-linked SSR markers. Fourteen polymorphic markers amplified 33 alleles across nine chromosomes, with 2-3 alleles per locus (average 2.36), indicating moderate genetic variability. High PIC, resolving power (Rp), effective multiplex ratio (EMR), and marker index (MI) values were observed for markers Xwmc603, Xwmc161 and Xgwm577, demonstrating strong discriminatory ability. Genetic similarity based on Jaccard's coefficient ranged from 0.36 to 0.88. UPGMA cluster analysis grouped the genotypes into two major clusters, with three genotypes in cluster I and seventeen in cluster II, confirming sufficient molecular diversity for selecting divergent parents to improve terminal heat tolerance in wheat.

Erjigitov *et al.* (2025) evaluated heat tolerance in six wheat genotypes using phenotypic and molecular approaches under optimal (25°C) and heat stress (35°C) conditions. Significant variation was observed for seedling and root length, with KR12-5003 showing high tolerance based on seedling length (HTI = 71.8%) and moderate tolerance for root length (HTI = 36.6%). Molecular analysis using 40 SSR markers (24 polymorphic) detected 87 alleles with a mean value of 3.6 alleles per locus (PIC: 0.01–0.81; He: 0.05–0.83), indicating substantial genetic diversity. Cluster analysis revealed that KR12-5003 was genetically distinct from other genotypes, suggesting the presence of unique alleles associated with heat tolerance.

Kumari *et al.* (2025) assessed genetic diversity among 10 bread wheat genotypes using Simple Sequence Repeat (SSR) markers alongside fifteen morpho-physiological traits. All ten SSR primers showed polymorphic amplification, generating altogether 64 polymorphic bands. The polymorphism information content values ranged from 0.39 for primer GWM 160 to 0.92 for XGWM 132. Allele numbers at each locus ranged from one (GWM 160 and GWM 165) to four (WMS 5, XGWM 484, and XGWM 132). A positive association was observed between PIC values and repeat motif variability across SSR loci, with primers containing more

complex repeat motifs such as WMS 5 {(TC)n(T)n(GT)n(GA)n} and XGWM 132 {(GA)n(GAA)n} exhibiting higher allelic diversity. The genotypes were classified into two major clusters using the UPGMA method, which were further subdivided into four sub-clusters based on genetic similarity coefficients.

13. Materials and methods:

The experimental materials will be comprised of 90 bread wheat genotypes, which will be obtained from Wheat Research Station, JAU, Junagadh.

13.1	**Centre of Experiment**	**:**	1. Sagdividi Farm, Department of Seed Science and Technology, Junagadh Agricultural University, Junagadh. 2. Main Dry Farming Research Station, Junagadh Agricultural University, Targhadia.
13.2	**Year and season of experiment**	**:**	The evaluation of 90 genotypes will be carried out in the field during *Rabi* 2025-26 under timely sown (3rd week of November) and late sown (3rd week of December) conditions at each location (total four environments)
13.3	**Experimental design**	**:**	Randomized Block Design (RBD)
13.4	**Number of replications**	**:**	3 (Three)
13.5	**Spacing**	**:**	22.5 cm
13.6	**Plot size**	**:**	Single row plot of 4 meter length
13.7	**Molecular analysis**	**:**	Heat tolerance specific SSR markers (>30) will be identified from review of literature and will be utilized in the present study. Molecular work will be carried out in the laboratory of Department of Biotechnology, College of Agriculture, JAU, Junagadh
13.8	**Number of genotypes**	**:**	90 (As per Table 1)

Table 1 List of genotypes to be used in research

Sr. No.	Genotypes	Sr. No.	Genotypes	Sr. No.	Genotypes
1	BFKW 07	31	IIWBR DN-502	61	WCF 12-208
2	BNSR 6	32	IIWBR PHY 2	62	WCF 12-61
3	CG 1029	33	K 1616	63	WH 1402
4	CG 1036	34	KARAN POSHAN 2	64	WH 1403
5	DBW 187	35	KRL-210	65	WH-1306
6	DBW 824	36	LOK-01	66	GW 366
7	GJW-463	37	LOK-83	67	GW-11
8	GW-957	38	LOK-86	68	LBP 2017-2
9	GW-451	39	MACS 6768	69	RW 5
10	GW-173	40	MACS 6795	70	RWP 2017-21
11	GW-499	41	MP 1323	71	DWAP 1608
12	GW-513	42	MP 1378	72	RWP 2018-32
13	GW-543	43	MP-3288	73	DBW 166
14	GW-556	44	NIAW 4028	74	DTW 119
15	GW-567	45	PBW 766	75	HI 1674
16	HD 2967	46	PBW 803	76	HD 3428
17	HD 3386	47	PBW 835	77	DBW 386
18	HD 3407	48	PBW 891	78	AKAW 5100
19	HD 3437	49	PHSL 10	79	HD 3471
20	HD 3471	50	QLD 118	80	NWS 2222
21	HI 1655	51	QLD 122	81	DBW 443
22	HI 1668	52	RAJ 4541	82	HI 1669
23	HPW 484	53	RAJ-3077	83	LOK 79
24	HPW 487	54	RWP 2018-31	84	HI 1625
25	HPW 489	55	UAS 3022	85	HI 1544
26	HPW 493	56	UNNAT PBW 550	86	CG 1044
27	HS 545	57	VL 3024	87	WH 730
28	HS 628	58	VL 3035	88	NIAW 4120
29	HS 679	59	WAP 2320	89	NIAW 4114
30	HS 681	60	WAP 2321	90	WH 1306

13.9 Observations to be recorded

For each genotype in every replication, five healthy and competitive plants will be randomly selected to record observations on various characters, except for days to 50% heading, days to anthesis, grain filling period and days to maturity, which will be recorded on a plot basis. Mean values of all recorded observations will be used for subsequent statistical analysis. The following methodology will be followed for data recording.

13.9.1 Days to 50 per cent heading

Total number of days from the date of sowing to 50 per cent emergence of main spike in the plot will be counted and expressed as days to 50 per cent heading.

13.9.2 Days to anthesis

Number of days from the date of sowing to date of anthesis (fully open and functional flower) in the plot will be counted and expressed as days to anthesis.

13.9.3 Days to maturity

Number of days from date of sowing to the time when more than 80 per cent of spikes in the plots turned green yellow will be recorded.

13.9.4 Grain filling period

Number of days from anthesis to grain maturity will be counted on plot basis.

13.9.5 Number of productive tillers per plant

Number of tillers bearing spike will be counted at the time of maturity.

13.9.6 Plant height (cm)

The height of plant will be measured in centimeters from the base of the plant to the tip of the main spike (excluding awns) at the time of maturity.

13.9.7 Spike length (cm)

Length from base to tip of the main spike (excluding awns) will be measured in centimeters at the time of maturity.

13.9.8 Number of spikelets per main spike

The number of spikelets in main spike will be counted at the time of harvest.

13.9.9 Number of grains per main spike

Number of grains per main spike will be counted from main spike of individual selected plants after harvesting and threshing.

13.9.10 1000-grain weight (g)

Random sample of 1000 grains per genotype from each replication will be counted and recorded in grams.

13.9.11 Grain yield per plant (g)

Total grains harvested from individual selected plants will be weighted in grams and averaged.

13.9.12 Biological yield per plant (g)

After harvesting and drying of plant, total biological yield per plant will be determined in grams by addition of total dry weight of plant and grain yield per plant.

13.9.13 Harvest index (%)

Harvest index will be calculated in percentage by using the following formula:

$$\text{Harvest index (\%)} = \frac{\text{Grain yield per plant (g)}}{\text{Biological yield per plant (g)}} \times 100$$

13.9.14 Flag leaf area (cm^2)

The flag leaf area will be measured of flag leaf of five randomly selected plants using a leaf area meter.

13.9.15 SPAD-Chlorophyll Meter Reading (SCMR-reading)

The SCMR reading will be recorded from third fully expanded leaf of randomly selected five plants with the help of chlorophyll meter SPAD-502 plus (Konica Minolta Sensing, Inc., Spectrum Technologies, Inc. SN: 20002897) at 75 days after sowing.

13.9.16 Canopy temperature depression (CTD):

CTD is the difference between canopy temperature and air temperature. The measurements will be taken by infrared thermometer (Fluke 63, IR Thermometer, Fluke Corporation, Everett, USA with laser pointer facility). The measurement will be taken at a distance of 1 meter and at an angle of approximately 45°. CTD readings will be recorded during clear, sunny days at the grain filling stage, preferably between 12:00 PM and 2:00 PM, when temperature stress is maximum, to ensure accurate assessment of genotypic variation under heat stress conditions.

13.9.17 Heat susceptibility index (HSI)

The heat susceptibility index (HSI) will be estimated for grain yield using following formula suggested by Fischer and Maurer (1978).

$$\text{HSI} = \frac{1-(YL-YN)}{1-(XL-XN)}$$

Where,

YL = Mean grain yield (g) of a genotype under late sown conditions

YN = Mean grain yield (g) of a genotype under normal sown conditions

XL= Mean grain yield (g) of all genotypes under late sown conditions

XN = Mean grain yield (g) of all genotypes under normal sown conditions

13.9.18 Yield stability ratio (YSR)

The yield stability ratio (YSR) will be calculated as per formula given by Lewis (1954).

$$YSR = \frac{\text{Grain yield under late sown condition (g)}}{\text{Grain yield under normal sown condition (g)}} \times 100$$

14. Statistical analysis

Replication-wise mean values of each genotype and environment for various characters under study will be utilized for statistical and genetical analysis. Statistical procedures used for analysis will be divided into following sub heads and explained accordingly.

14.1 Analysis of variance for experimental design

Analysis of variance suggested by Snedecor and Cochran (1989) and reviewed by Panse and Sukhatme (1995) will be followed to test the significance of differences among the genotypes for all the characters under study.

14.2 Variability parameters

Genotypic, phenotypic and environmental variance will be calculated as per the formula suggested by Johnson *et al*. (1955). Phenotypic coefficient of variation and genotypic coefficient of variation will be calculated as per the formula suggested by Burton (1952). Heritability and genetic advance will be estimated using the formula suggested by Allard (1960).

14.3 Correlation coefficients

Phenotypic and genotypic correlation coefficients for all the pair wise characters will be worked out as per Al-Jibouri *et al*. (1958).

14.4 Path coefficient analysis

Path coefficient analysis will be performed following the procedure suggested by Dewey and Lu (1959).

14.5 Genetic divergence

Genetic divergence will be estimated based on the procedure suggested by Mahalanobis (1936) and grouping of clusters following the Tocher method described by Rao (1952).

14.6 Selection indices

Selection indices will be constructed based on the method described by Smith (1936).

14.7 Genotype × environment interaction and stability parameters

Genotype × environment interaction and stability analysis will be carried out according to either Eberhart and Russell (1966) model or AMMI model (Farshadfar *et al.*, 2011) by using R studio or other suitable software.

14.8 Molecular diversity using microsatellite markers

DNA will be extracted from newly expanded fresh leaves using Cetyl Trimethyl Ammonium Bromide (CTAB) protocol with some modifications as described by Doyle and Doyle (1990). Cluster analysis using molecular markers data will be generated through UPGMA (Unweighted Pair Group Method using Arithmetic means) algorithm by NTSYSpc (Numerical Taxonomy System) version 2.02 or other appropriate software. The banding pattern of SSR will be analyzed by Jaccard's similarity coefficients.

15. References

Alexander, L. V., Zhang, X., Peterson, T. C., Caesar, J., Gleason, B., Tank, A., Haylock, M., Collins, D., Trewin, B., Rahimzadeh, F., Tagipour, A., Kumar, K. R., Revadekar, J., Griffiths, G., Vincent, L., Stephenson, D. B., Burn, J., Aguilar, E., Brunet, M., Taylor, M., Zhai, P., Rusticucci, M. and Vazquez-Aguirre, J. L. (2006). Global observed changes in daily climate extremes of temperature and precipitation. *Journal of Geophysical Research*, **111(D5)**: 1-22.

Al-Jibouri, H. A., Miller, P. A. and Robinson, H. F. (1958). Genotypic and environmental variances in upland cotton cross of inter specific origin. *Agronomy Journal,* **50(10)**: 633-635.

Allard, R. W. (1960). *Principles of Plant Breeding,* John Willey and Sons*,* New York.

Anonymous. (2024). Global Production. https://fas.usda.gov/data/production?commodity=wheat Accessed on October 28, 2024.

Anonymous. (2025). Year and State wise Area, Production and Yield of Foodgrains – Wheat. https://dataful.in/datasets/21402/ Accessed on July 25, 2025.

Arunachalam, V. (1981). Genetic distance in plant breeding. *Indian Journal of Genetics,* **41(2)**: 226-236.

Asseng, S., Ewert, F., Martre, P., Rötter, R. P., Lobell, D. B., Cammarano, D. and Zhu, Y. (2015). Rising temperatures reduce global wheat production. *Nature Climate Change,* **5(2)**, 143-147.

Balla, K., Karsai, I., Bencze, S. and Veisz, O. (2012). Germination ability and seedling vigour in the progeny of heat-stressed wheat plants. *Acta Agronomica Hungarica*, **60(4)**: 299-308.

Baranwal, D., Kumar, R., Azad, C. and Munjal, R. (2024). Assessment of terminal heat tolerance among wheat (*Triticum aestivum*) genotypes based on multivariate analysis and selection indices. *Indian Journal of Agricultural Sciences,* **94(9)**: 923-928.

Bhandari, R. and Poudel, M. R. (2024). Genotype × environment interaction and selection parameters for high yielding wheat genotypes under irrigated and heat stress environment. *Journal of Sustainable Agriculture and Environment*, **3(1)**: e12098.

Bhutto, A. H., Rajpar, A. A., Kalhoro, S. A., Ali, A., Kalhoro, F. A., Ahmed, M., Raza, S. and Kalhoro, N. A. (2016). Correlation and regression analysis for yield traits in wheat (*Triticum aestivum* L.) genotypes. *Natural Science,* **8(3)**: 96-104.

Burton, G. W. (1952). Quantitative inheritance in grasses. *Proceedings of 6th International Grassland Congress, Pennsylvania State College*, **1**: 277-283.

Chandramaniya, C. P., Kale, B., Bhattacharya, S., Patel, D. S. and Makwana, D. V. (2025). Genetic architecture for heat tolerance in bread wheat (*Triticum aestivum* L.). *Plant Archives*, **25(Supplement 1)**: 128-135.

Chandramaniya, C. P., Dhairya V. Makwana, Himani P. Vadodariya, Divya S Patel, Sampurna Bhattacharya, Parmar Mukeshkumar and Bhushan Kale. 2025. "Selection Indices for Heat Tolerance Improvement in Bread Wheat". *Journal of Advances in Biology & Biotechnology*, **28(7):**1182-92.

Chandramaniya, C. P., Kale, B., Bhattacharya, S., Modha, K., kumar Patel, V., Vekariya, R. D. and Savaliya, D. Validation of SSR markers linked to heat tolerance in bread wheat genotypes. *Plant Science Today*, **12(3):**1-10.

Das, N. R. (2008). *Wheat Crop Management*. Scientific Publishers, Jodhpur, India.

Desheva, G. (2025). Effects of genotype, environment and their interaction on quality characteristics of winter bread wheat. *Journal of Basic and Applied Research in Biomedicine*, ***2*(3):** 363-372.

Detroja, H. S., Rathod, V. B., Chaudhary, J. H., Pansuriya, A. G., Raval, L. J. and Dangariya, P. P. (2024). Selection indices in bread wheat (*Triticum aestivum* L.). *International Journal of Advanced Biochemistry Research,* **8(12)**: 939-941

Devesh, P., Moitra, P. K. and Shukla, R. S. (2021). Correlation and path coefficient analysis for yield, yield components and quality traits in wheat. *Electronic Journal of Plant Breeding,* **12(2)**: 388-395.

Devi, S., Singh, V., Yashveer, S., Dalal, M. S., Chawla, R., Akbarzai, D. K. and Chaurasia, H. 2023. Molecular characterization of bread wheat (*Triticum aestivum*) genotypes using SSR markers. *Indian Journal of Agricultural Sciences,* **93(9)**: 948-953.

Dewey, D. R. and Lu, K. H. (1959). A correlation and path coefficient analysis of components of crested wheat grass seed production. *Agronomy Journal,* **51(9)**: 511-518.

Doyle, J. J. and Doyle, J. L. (1990). Isolation of plant DNA from fresh tissue. *Focus*, **12(1)**: 13-15.

Dubey, R.; Pathak, H., Chakrabarti, B., Singh, S., Gupta, D. K. and Harit, R. C. (2020). Impact of terminal heat stress on wheat yield in India and options for Adaptation. *Agricultural Systems* **181**: 102826.

Eberhart, S. A. and Russell, W. A. (1966). Stability parameters for comparing the varieties. *Crop Science*, **6(1)**: 36-40.

Erjigitov, D. S., Turaev, O. S., Dolimov, A. A., Mamatkulova, G. F., Mukhammadiev, O. A., Tursunmurodova, B. T. and Kushanov, F. N. (2025). Molecular Characterization of heat tolerance in bread wheat (*Triticum aestivum* L.) cultivars/lines using genomic SSR markers. *Journal of Plant Interactions*, **20(1)**: 2546997.

Farhad, M., Hasan, M. M., Hossain, M. A., and Rahman, M. A. (2023). Impact of heat stress on wheat yield and adaptation strategies: A review. *Frontiers in Sustainable Food Systems,* **7**, 1203721.

Farshadfar, E., Mahmodi, N. and Yaghotipoor, A. (2011). AMMI stability value and simultaneous estimation of yield and yield stability in bread wheat (*Triticum aestivum* L.). *Australian Journal of Crop Science,* **5(13)**: 1837-1844.

Fernandez, G. C. J. (1991). Analysis of genotype × environment interaction by stability estimates. *HortScience*, **26(8)**: 947-950.

Fischer, R. A. and Maurer, R. (1978). Drought resistance in spring wheat cultivar. I. Seed yield responses in spring wheat. *Australian Journal of Agricultural Research*, **29**: 892-912.

Fisher, R. A. (1936). The use of multiple measurements in taxonomic problems. *Annals of Eugenics*, **7**: 179-188.

Fisher, R. A. and Byerlee, D. R. (1991). Trends of wheat production in the warmer area: Major issues and economic consideration. *In Wheat for the Non-Traditional Warm Areas, Proceedings of Conference*, Iguazu, 29 July-3 August, 1990, pp. 3-27.

Fokar, M., Blum, A. and Nguyen, H. T. (1998). Heat tolerance in spring wheat. II. Grain filling. *Euphytica*, **104(1)**: 9-15.

Gauch, H. G. (1988). Model selection and validation for yield trials with interaction. *Biometrics,* **44(3)**, 705-715.

Gauch, H. G. and Zobel, R. W. (1996). AMMI analysis of yield trials. In M. S. Kang and H. G. Gauch (Eds.), *Genotype by environment interaction* (pp. 85–122).

Gerema, G., Lule, D., Lemessa, F. and Mekonnen, T. (2020). Morphological characterization and genetic analysis in bread wheat germplasm: A combined study of heritability, genetic variance, genetic divergence and association of characters. *Agricultural Science and Technology,* **12(4)**: 301-311.

Haque, M. S., Saha, N. R., Islam, M. T., Islam, M. M., Kwon, S. J., Roy, S. K. and Woo, S. H. (2021). Screening for drought tolerance in wheat genotypes by morphological and SSR markers. *Journal of Crop Science and Biotechnology*, **24(1)**: 27-39.

Hays, D., Mason, E., Hwa, D. J., Menz, M. and Reynolds, M. (2007). Expression quantitative trait loci mapping heat tolerance during reproductive development in wheat (*T. aestivum*). In: Buck, H. T., Nisi, J. E. and Salomon, N. (Eds.), *Wheat Production in Stressed Environments*, Springer, Amsterdam, pp. 373-382.

*Hotelling, H. (1936). Relations between two sets of variates. *Biometrika*, **28(3/4)**: 321- 377.

Howard, A. (1924). *Crop Production in India: A Critical Survey of Its Problems*. Oxford University Press, Oxford, UK, p. 156.

Johnson, H. W., Robinson, H. F. and Comstock, R. E. (1955). Estimates of genetic and environmental variability in soybean (*Glycine max*). *Agronomy Journal*, **47**: 314-318.

Joshi, A. K., Chand, R., Arun, B., Singh, R. P. and Ortiz, R. (2007). Breeding crops for reduced tillage management in the intensive rice wheat systems of south Asia. *Euphytica*, **153(1-2)**: 135-151.

Kajla, S. L., Sharma, A. K., Singh, H., Bhakal, M. and Natwaria, M. R. (2022). Heat susceptibility index analysis for terminal heat tolerance in bread wheat (*Triticum aestivum* L. em. Thell.). *The Pharma Innovation Journal,* **11(2)**: 942-946.

Karkaji, F. A., Hervan, E. M., Roustaii, M., Bihamta, M. and Mohammadi, S. (2023). Comprehensive stability analysis of wheat genotypes through multi-environmental trials. *Journal of Agricultural Sciences*, **29(1)**: 317-334.

Khare, V., Shukla, R.S., Pandey, S., Singh, S. K. and Singh, C. (2024). Exploring the genotype-environment interaction of bread wheat in ambient and high temperature planting conditions: A rigorous investigation. *Scientific Reports*, **14**: 2402.

Kiranakumara, D. M., Biradar, S. S., Aishwarya, B., Akshaya, M. and Swaroop, K. O. (2024). Assessment of genetic variability, heritability and correlation in advanced breeding lines of bread wheat (*Triticum aestivum* L.) under heat stress condition. *Electronic Journal of Plant Breeding*, **15(3)**: 632-641.

Kumar, S., Singh, G. P. and Prasad, P. V. V. (2023). Heat stress in wheat: Impacts, adaptation, and mitigation strategies in South Asia. *Journal of Agronomy and Crop Science,* **209(4)**, 789-805.

Kumar, J., Yadav, V. K., Yadav, R. K., Singh, S. V., Maurya, C. L. and Kumar, A. (2024). Estimation of heritability, genetic advance and correlation in bread wheat (*Triticum aesitvum* L.) under heat stress condition. *Journal of Advances Biology and Biotechnology*, **27(10)**: 82-93.

Kumari, M., Sharma, H., Dadeech, A. and Dashora, A. (2025). Molecular characterization and genetic diversity assessment of bread wheat [*Triticum aestivum* (L.) em. Thell] genotypes using SSR markers. *International Journal of Current Microbiology and Applied Sciences*, **14(5)**: 139-147.

Labonno, S., Raihan, M. and Mohi-Ud-Din, M. (2025). Morpho-Physiological Indices for Identification of Heat Tolerant Wheat Genotypes. *Phyton*, **94(5):** 1545.

Legesse, B. W., Myburg, A. A., Pixley, K. V. and Botha, A. M. (2007). Genetic diversity of African maize inbred lines revealed by SSR markers. *Hereditas,* **144**: 10-17.

*Lewis, F. B. (1954). Gene-environment interaction: A relationship between dominance, heterosis, phenotypic stability and variability. *Heredity*, **8**: 333-356.

Lupton, F. G. H. (1987). *Wheat Breeding: Its Scientific Basis*. Chapman and Hall Ltd., London.

Mahalanobis, P. C. (1936). On the generalized distance in statistics. *Proceedings. of the National Institute of Sciences of India*, **2(1)**: 49-55.

Mahpara, S., Imran, M., Khalid, M. A., Fatima, N., Ammar, A., Liaqat, S. and Kanwal, S. (2024). Correlation and path analysis in different wheat genotypes for grain yield and its related traits. *Pakistan Journal of Scientific and Industrial Research*, **67B(3)**: 226-231.

Mangroliya, U. C., Patel, H. R. and Parmar, N.A. (2024). Formulation of selection indices for yield enhancement in bread wheat (*Triticum aestivum* L.). *Annals of Arid Zone*, **62(2)**: 109-114.

Mondal, S., Singh, R. P., Mason, E. R., Huerta-Espino, J., Autrique, E. and Joshi, A. K. (2013). Grain yield, adaptation and progress in breeding for early maturing and heat-tolerant wheat lines in South Asia. *Field Crops Research,* **146,** 73-81.

Mullualem, D., Tsega, A., Mengie, T., Fentie, D., Kassa, Z., Fassil, A. and Astatkie, T. (2024). Genotype-by-environment interaction and stability analysis of grain yield of bread wheat (*Triticum aestivum* L.) genotypes using AMMI and GGE biplot analyses. *Heliyon*, **10(12)**: e32918.

Nagarajan, S. (2005). Can India produce enough wheat even by 2020. *Current Science,* **89(9)**: 1467-1471.

Panse, V. G. and Sukhatme, P. V. (1995). *Statistical Methods for Agricultural Workers.* Indian Council of Agricultural Research, New Delhi.

Patel, N. S. and Raval, L. J. (2018). Selection indices for yield improvement in bread wheat (*Triticum aestivum* L.) under late sown condition. *J. Pharmacognosy and Phytochemistry,* **7(5)**: 1586-1588.

Patel, N. S., Patel, J. B. and Tomar, R. S. (2024). Identification of heat tolerant bread wheat (*Triticum aestivum* L.) genotypes through heat susceptibility index (HSI) and SSR markers. *Cereal Research Communications*, **52(2)**: 465.

Patel, N. S., Patel, J. B., Shekhawat, V. S. and Kumar, R. (2023). Stability analysis for grain yield and its components in bread wheat (*Triticum aestivum* L.). *Agricultural Mechanization in Asia*, **53(7)**: 9781-9797.

*Pearson, K. (1926). On the coefficient of racial likeness. *Biometrika*, **18**: 105-117.

Peto, R. (1982). Statistical aspects of cancer trials. In: *Treatments of Cancer* (K.E. Hainan, Ed.). Chapman and Hall, London, pp. 867-871.

Poudel, M. R., Lamsal, N., Rijal, A., Ghimire, P., Ghimire, M., Kafle, K., Lamsal, K. and Bhusal, P. (2024). Correlation and path coefficient analysis in wheat (*Triticum aestivum* L.) under heat stress condition. *Cornous Biology*, **2(2)**: 9-14.

Poudel, M. R., Poudel, P. B., Puri, R. R. and Paudel, H. K. (2021). Variability, correlation and path coefficient analysis for agro-morphological traits in wheat genotypes (*Triticum aestivum* L.) under normal and heat stress conditions. *International Journal of Applied Sciences and Biotechnology,* **9(1)**: 65-74.

Qi-Lun, Y., Ping, F., Ke-Cheng, K. and Guang-Tang, P. (2008). Genetic diversity based on SSR markers in maize (*Zea mays* L.) landraces from Wuling mountain region in China. *Journal of Genetics,* **87**: 287-291.

Rajput, A. G., Misal, A. M., Jadhav, A. V., Shinde, S. A. and Rathod, A. H. (2025). Correlation and path coefficient analysis in bread wheat (*Triticum aestivum* L.). *International Journal of Agriculture and Food Science,* **12(3)**: 145-152.

Rane, J. and Nagarajan, S. (2004). High temperature index-for field evaluation of heat tolerance in wheat varieties. *Agricultural Systems*, **79(2)**: 243-255.

Rao, C. R. (1952). Advanced *Statistical* Methods *in* Biometric *Research*, John Willey & Sons, New York.

Reddy, S. G. P. and Babariya, C. A. (2020). Selection indices for yield improvement in bread wheat (*Triticum aestivum* L.). *Electronic Journal of Plant Breeding,* **11(1)**: 314-317.

Regmi, S., Poudel, B., Oja, B. R., Kharel, R., Joshi, P., Khanal, S. and Kandel, B. P. (2021). Estimation of genetic parameters of different wheat genotype traits in Chitwan, Nepal. *International Journal of Agronomy,* **2021(1)**: 6651325.

Rehman, H. U., Tariq, A., Ashraf, I., Ahmed, M., Muscolo, A., Basra, S. M. A. and Reynolds, M. (2021). Evaluation of physiological and morphological traits for improving spring wheat adaptation to terminal heat stress. *Plants*, **10**: 455.

Reynold, M. P. (2010). *Climate Change and Crop Production. CABI Climate Change Series.* CAB International, Oxfordshire, UK, p. 292.

Santosh and Jaiswal, J. P. (2024). Assessment of genetic diversity for terminal heat tolerance in bread wheat (*Triticum aestivum* L. em. Thell.) under very late sown condition. *International Journal of Research in Agronomy*, **SP-7(7)**: 14-20.

Santosh, Jaiswal, J. P., Singh, A. and Gahatyari, N. C. (2019). Assessment of genetic diversity for terminal heat tolerance in bread wheat (*Triticum aestivum* L. em. Thell.). *Journal of Pharmacognosy and Phytochemistry*, **8(1)**: 2572-2579.

Shah, S., Mehta, D. R. and Raval, L. J. (2016). Selection indices in bread wheat (*Triticum aestivum* L.). *Electronic Journal of Plant Breeding,* **7(2)**: 459-463.

Sharma, I. and Sendhil, R. (2016). Wheat Production in India - A Decadal Synopsis http://www.FnBnews.com, Accessed on November 22, 2024.

Smith, H. F. (1936). A discriminant function for plant selection. *Annals of Eugenics,* **7**: 240- 250.

Snedecor, G. W. and Cochran, W. G. (1989). *Statistical Methods.* 8th Ed. Iowa State University Press, Ames.

Tuhin, M. M. H., Rahman, M. A. and Hasan, M. M. (2025). Climate change-induced heat stress effects on wheat production and potential mitigation strategies. *Environmental and Experimental Botany,* **220**, 105678.

Turkoglu, A., Haliloglu, K., Mohammadi, S. A., Ozturk, A., Bolouri, P., Ozkan, G. and Jamshidi, B. (2023). Genetic diversity and population structure in turkiye bread wheat genotypes revealed by simple sequence repeats (SSR) markers. *Genes*, **14(6)**: 1182.

Van Inghelandt, D., Melchinger, A. E.,Lebreton, C. and Stich, B. (2010). Population structure and genetic diversity in a commercial maize breeding program assessed with SSR and SNP markers. *Theoretical and Applied Genetics,* **120**:1289-1299.

Wardlaw, I. F. (1994). The effect of high temperature on kernel development in wheat: Variability related to pre-heading post-anthesis conditions. *Australian Journal of Plant Physiology,* **21(6)**: 731-739.

Wardlaw, I. F., Dawson, I. A. and Munibi, P. (1989). The tolerance of wheat to high temperatures during reproductive growth. 2. Grain development. *Australian Journal of Agricultural Research,* **40**: 15-24.

Wright, S. (1921). Correlation and causation. *Journal of Agriculture Research,* **20(7)**: 557-585.

Yadav, S. B. K. and Bhatt, S. (2024). Heat stress and its tolerance in wheat. *Cogent Food and Agriculture,* **10(1)**: 2413398.

Yagdi, K. (2002). Research on determination of stability parameters of bread wheat (*Triticum aestivum* L.) cultivars and lines grown in Bursa province. *Journal of Agricultural Faculty of Uludag University,* **16(1)**: 51-57.

Yasin, B. A., Shubhra, S., Abdu, M., Shiferaw, G., Teshome, D., Gudina, G., Girshe, L. and Dugasa, T. (2024). Assessing genetic diversity in bread wheat (*Triticum aestivum* L.) using D^2 statistical analysis. *Asian Journal of Dairy and Food Research.* **DR-2139**: 1-6.

Zobel, R. W., Wright, M. J. and Gauch, H. G. (1988). Statistical analysis of a yield trial. *Agronomy Journal,* **80(3)**: 388-393.

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